APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2009, p. 7287–7290 Vol. 75, No. 22
0099-2240/09/$12.00 doi:10.1128/AEM.00915-09

Dominant Bacteria and Biomass in the Kuytun 51 Glacier▽†

Shu-Rong Xiang,[1,2]* Tian-Cui Shang,[3] Yong Chen,[4] Ze-Fan Jing,[2] and Tandong Yao[1,2]

Key Laboratory of Tibetan Environment Changes and Land Surface Processes, Institute of Tibetan Plateau Research, Chinese Academy of Science, Beijing 100085, China[1]; State Key Laboratory of Cryospheric Science, Cold and Arid Regions Environmental Engineering Research Institute, Chinese Academy of Science, Lanzhou, Gansu 730000, China[2]; Department of Plant Pathology, Gansu Agricultural University, Lanzhou, Gansu 730070, China[3]; and School of Life Science, Lanzhou University, Lanzhou, Gansu 730000, China[4]

Received 22 April 2009/Accepted 4 September 2009

Dominant bacteria in the different habitats in the Kuytun 51 Glacier were investigated using a 16S rRNA gene clone library sequencing technique. Results showed diverse bacteria on the glacial surface, with the dominant phyla being *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*. UniFrac data showed distinct community patterns between the Kuytun and Himalayan Rongbuk glaciers.

Comparisons of geographically distinct glaciers worldwide have shown a great variation in microbial biomass and community structure (6, 8, 15, 18, 22, 34, 36). The variability is largely controlled by climatic and environmental factors, including geographic location (4, 12, 19, 24), wind direction, wind speed, light intensity, and availability of nutrients and liquid water (5, 6, 9, 13, 19, 24). There is some limited evidence of biogeographic effects on the distribution of microorganisms in the geographically different glaciers (7, 15, 25, 34, 36). However, main factors driving the dynamics of microbial community in glacial systems remain unclear.

The Kuytun 51 Glacier is a typical cold-based subcontinental glacier (11). The microbial communities in subcontinental glaciers have not been studied in detail, as there have been only a few reports on continental and polar glaciers (7, 16, 22, 23). To initially investigate the biogeographic effects on the distribution of microorganisms in glacial ice, six 16S rRNA gene libraries were established from the Kuytun 51 Glacier. Bacterial-community comparison was conducted among the Kuytun 51, John Evans (Canada) (22), Alaska Bench (22), and Himalayan Rongbuk (16) glaciers.

The glacial samples were obtained with extreme caution from the different habitats, solid ice, firn, and snow, at the terminus, middle, and top of the Kuytun 51 Glacier (84°24′E, 43°43′N) in the Tianshan Mountains in 2005. Numbers of live cells and total biomass in the melt water were determined with a flow cytometer in combination with cell marker propidium iodide and carboxyfluorescein diacetate by following the method of Amor et al. (3), except for cell staining for 15 min at 25°C. DNA nucleic acids were extracted from a portion (400 ml) of the melt water filtered through a sterile 0.22-μm-pore-size filter unit (Millipore) by following the protocols described by Zhou et al. (38). A neighbor-joining (28) phylogeny for the ClustalX (30) aligned sequences was constructed using MEGA

TABLE 1. Bacterial biomasses in the different habitats of the Kuytun 51 Glacier

Zone	Altitude (m above sea level)	Description	Total no. of cells (10^4 cells ml^{-1})	No. of live cells (10^2 cells ml^{-1})	% of cells that were live
Dry snow	3,725		16.96	11.45	0.68
Firn	3,601	Firn zone[a]	105.40 ± 23.54	232.34 ± 36.37	2.20 ± 0.88
		White	81.86 ± 20.36	195.97 ± 30.78	2.39 ± 1.20
		Brown	128.93 ± 27.14	268.71 ± 40.32	2.08 ± 0.32
Ablation	3,505	Ablation zone[b]	34.92 ± 24.59	77.72 ± 51.26	2.22 ± 0.33
		Ice, H_2O, and particles	43.18	94.9	2.2
		Ice	54.32	118.18	2.18
		H_2O	7.27	20.07	2.76

[a] The average value was calculated by the analysis of three white and three brown snow samples collected in the firn zone of the glacial surface.
[b] The average value was calculated by the analysis of ice, ice-water particles, and water samples collected in the ablation zone.

* Corresponding author. Mailing address: Institute of Tibetan Plateau Research, Chinese Academy of Science, Beijing 100085, China. Phone: 86-10-6284-9367. Fax: 86-10-6284-9886. E-mail: srxiang@ns.lzb.ac.cn.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 11 September 2009.

TABLE 2. Biomass comparison among ice samples from the Kuytun 51 Glacier and other glaciers worldwide

Glacier	Location	Altitude (m above sea level)	Glacial type	Total biomass (10^4 cells/ml)	Live biomass[a]	Reference
Kuytun Tienshan Mt.	84°24′E 43°43′N	3,500–4,000	Subcontinental	7.3–129	11.4×10^2–269×10^2 cells/ml	This study
Rongbuk Hymalaya	28°01′N 86°57′E	6,000–8,000	Continental	2.1–90.4	0.1–5 CFU/ml	16, 34
Guoqu Geladandong	33°34′N 91°10′E	6,621	Continental	0.3–83 (ice core)	ND	15
Palong	29°14′N 96°55′E	4,618–5,974	Marine	0.68–37.2	ND	15
Kongsvege	78°56′N 11°52′E			1–20		2
Muztag Ata	38°17′N 75°04′E	6,350	Continental	0.2–21.7 (ice core)	0–1.04 CFU/ml	32
Guliya	35°21′N 81°31′E	6,200	Subcontinental	1 (ice core)	0.07–1.8 CFU/ml	8
Malan	35°50′N 90°40′E	5,680	Continent	0.005–0.04 (ice core)	<1–0.85 CFU/ml	35
Puruogangri	33°44′N 89°20′E	6,000	Subcontinental	2.56–25.5 (ice core)	0–7.6 CFU/ml	37
Bench (Alaska)	61.03°N 145.7°W	950–1,600	Subglacial	6.6–37	ND	22
John Evans (Arctic)	79°40′N 74°00′W	100–1,500	Polythermal	ND	1–10 CFU/ml	22
Antarctica	78°27′S 106°48′E	3,500	Polar	0.003–0.83		1
GISP2 Greenland	72°35′N 37°38′W	3,203	Polar	6,100–9,100 (base ice)	ND	18
South Pole	90°S	2,835	Polar	0.02–0.5		6
Taylor Dome, Antarctica	77°47′S 158°43′E	2,365	Polar		~10 CFU	7

[a] ND, no determination.

4.0 (27) with the maximum composite likelihood method (see the detailed methods in the supplemental material).

The Kuytun 51 Glacier contains a relatively large biomass, ranging from 7.27×10^4 to 1.29×10^6 cells/ml and 1.14×10^3 to 2.69×10^4 cells/ml for the total biomass and live biomass, respectively (Table 1); these numbers are higher than in most glaciers worldwide (Table 2). This could be attributed to the fact that Kuytun 51 Glacier is located near (within about 10 km of) human settlements and surrounded by vast deserts: the Gobi, Muyun Kum, Kyzyl Kum, and Kara Kum deserts (14). The frequent dust storm outbreaks that emerge in and around the arid and semiarid regions possibly carry a large number of microbes associated with dust onto the glacier via prevalent westerly winds (10, 14, 33). Biomass is generally higher in the mountainous glaciers than in the polar glaciers, which are far away and little influenced by the activities of human beings (Table 2) (8, 34). However, there are some exceptions, including low biomass in the Malan Glacier (35) and high biomass in the "silty" ice from the GISP 2 ice core (18). This may be attributed to the specific climatic and environmental conditions and local habitats. More data are necessary before definitive conclusions can be drawn because of different methods used in the estimation of biomass.

All 112 16S rRNA gene sequences obtained from the Kuytun 51 Glacier were >97% similar to known species from the different environments. They fell into 30 subphyla within 10 phyla: *Alpha-*, *Beta-*, *Delta-*, and *Gammaproteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Actinobacteria*, *Deinococcales*, and *Cyanobacteria* (see the supplemental material). There was an apparent difference in the phylogenetic distributions of bacteria in the glacier (Fig. 1). *Proteobacteria* were the most dominant bacteria, *Bacteroidetes* and *Cyanobacteria* were the second-most-common bacteria on the glacier surface, and *Actinobacteria* were present along the glacier surface, although

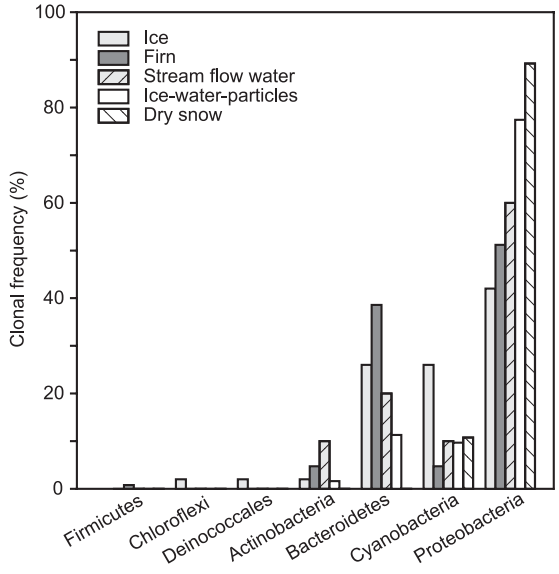

FIG. 1. Clonal frequency of the main phylogenetic groups based on the BLAST result of 16S rRNA gene clone sequencing in a single library.

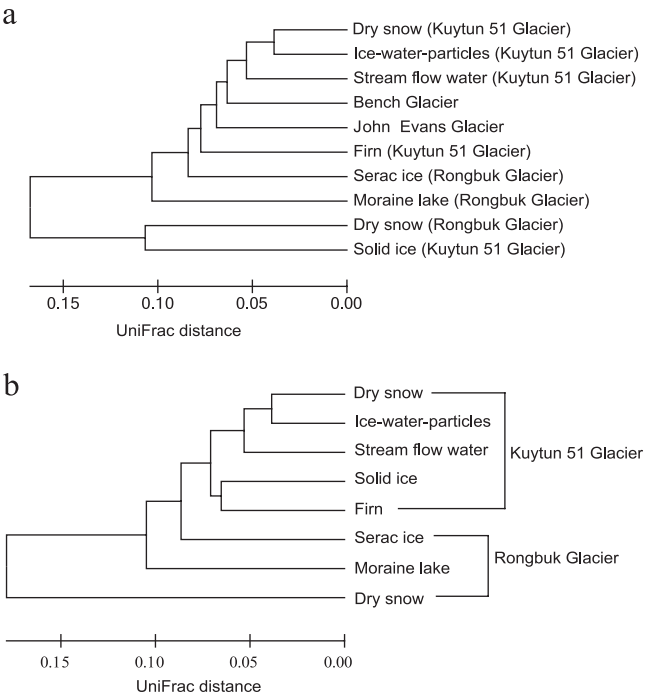

FIG. 2. Hierarchical clustering showing the overall phylogenetic distances between the bacterial communities on the Kuytun glacial surface (this study) and from John Evans Glacier, Canada (22), Alaska Bench Glacier (22), and East Rongbuk Glacier north of the Himalayas (16). (a) Comparison of communities among the Kuytun 51 Glacier, John Evans Glacier, Bench Glacier, and Rongbuk Glacier; (b) community comparison between the Kuytun 51 Glacier and Rongbuk Glacier (sequence KuyT-ice-3 with a later portion of the 16S rRNA gene was removed from the phylogenetic tree list). Distances were estimated with the weighted UniFrac algorithm (17). A sequence jackknifing technique was applied to each cluster to determine the sensitivity of the relationships to sample size.

they accounted for a small proportion of the total clones. Only a few clones belonging to the families *Chloroflexi* and *Deinococcales* and *Firmicutes* were found from the ice and firn niches.

The Kuytun Glacier bacteria represented two distinct metabolic types, including phototrophs and heterotrophs. *Cyanobacteria* were the dominant phototrophs across the glacial surface and closely related to the known species in four subgroups: *Phormidium*, *Pseudanabaena*, and *Oscillatoria* spp. and *Stephanopyxidaceae*. These phototrophic *Cyanobacteria* have frequently been reported from geographically different glaciers, such as the Alaska (26), Chile Tyndall (24), Svalbard (23), and Antarctic (7, 19, 29) glaciers. However, only one cyanobacterial clone has been reported from the Rongbuk Glacier (16). These results suggest that *Cyanobacteria* are very common in some glaciers but rare in other glaciers, which can be attributed to the possibly different sources of microbial loads on the glacier and the specific selection effect of local climatic and environmental conditions on microorganisms. The highly pigmented and structured biofilms may allow the *Cyanobacteria* to tolerate extreme environments and dominate in the glaciers (31).

Betaproteobacteria and *Bacteroidetes* are the most common phyla in the Kuytung 51 Glacier (Fig. 1), which is consistent with previous reports on the bacterial communities in glaciers (4, 15, 16, 23). This is possibly attributed to their tolerance to oligotrophic environments (21) and a wide spectrum of substrate range in low-nutrient media (20). *Bacteroidetes* are able to produce copious amounts of extracellular polymeric substances on surfaces and have high catabolic ability for complex and more recalcitrant organic matter (4).

Also of significance are the apparent differences in dominant bacterial community compositions among the geographically distinct glaciers around the world (Fig. 2a and b). When subjected to UniFrac analysis, the dominant bacterial communities from the same glacier form distinct clusters from other glaciers (Fig. 2b). This promotes the idea of biogeography as an important factor with regard to microbial community structure.

Nucleotide sequence accession numbers. The GenBank accession numbers of the cloned sequences described in this study are EU263676 to EU263787.

We extend our special thanks to Lizhe An and Bingliang Xu for giving support for the completion of laboratory analysis for this study. We also thank Trista Vick very much for her kind help with the improvement of the English in this paper. We thank all of the members of the Kuytun Glacier 51 expedition for help with field sample collection.

This work was supported by the NSF Project of China (grants 40471025 and 40571038).

REFERENCES

1. **Abyzov, S. S., I. N. Mitskevich, and M. N. Poglazova.** 1998. Microflora of the deep glacier horizons of central Antarctica. Microbiology **67:**66–73.
2. **Amato, P., R. Hennebelle, O. Magand, M. Sancelme, A. Delort, C. Barbante, C. Boutron, and C. Ferrari.** 2007. Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol. Ecol. **59:**255–264.
3. **Amor, K. B., P. Breeuwer, P. Verbaarschot, F. M. Rombouts, A. D. L. Akkermans, W. M. De Vos, and T. Abee.** 2002. Multiparametric flow cytometry and cell sorting for the assessment of viable, injured, and dead bifidobacterium cells during bile salt stress. Appl. Environ. Microbiol. **68:**5209–5216.
4. **Battin, T. J., A. Wille, B. Sattler, and R. Psenner.** 2001. Phylogenetic and functional heterogeneity of sediment biofilms along environmental gradients in a glacial stream. Appl. Environ. Microbiol. **67:**799–807.
5. **Bhatia, M., M. Sharp, and J. Foght.** 2006. Distinct bacterial communities exist beneath a high Arctic polythermal glacier. Appl. Environ. Microbiol. **72:**5838–5845.
6. **Carpenter, E. J., S. Lin, and D. G. Capone.** 2000. Bacterial activity in South Pole snow. Appl. Environ. Microbiol. **66:**4514–4517.
7. **Christner, B. C., B. H. Kvitko, and J. N. Reeve.** 2003. Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. Extremophiles **7:**177–183.
8. **Christner, B. C., E. Mosley-Thompson, L. G. Thompson, V. Zagorodnov, K. Sandman, and J. N. Reeve.** 2000. Recovery and identification of viable bacteria immured in glacial ice. Icarus **144:**479–485.
9. **Hill, T. C. J., K. A. Walsh, J. A. Harris, and B. F. Moffett.** 2003. Using ecological diversity measures with bacterial communities. FEMS Microb. Ecol. **43:**1–11.
10. **Husar, R. B., J. M. Prospero, and L. L. Stowe.** 1997. Characterization of tropospheric aerosols over ocean with the NOAA/AVIIRR optical thickness operational product: passive remote sensing of tropospheric aerosol and atmospheric corrections from the new generation of satellite sensors. J. Geophys. Res. **102:**16889–16909.
11. **Jing, Z.-F., B.-S. Ye, K.-Q. Jiao, and H.-A. Yang.** 2002. Surface velocity on the glacier no. 51 at Haxilegen of the Kuytun River, Tianshan Mountains. J. Glaciol. Geocryol. **24:**563–566.
12. **Kikuchi, Y.** 1994. *Glaciella*, a new genus of freshwater *Canthocampyidae* (*Copepoda Harpacticoida*) from a glacier in Nepal, Himalayas. Hydrobiology **192:**59–66.
13. **Kohshima, S.** 1994. Ecological characteristics of the glacier ecosystem. Jpn. J. Ecol. **44:**93–98.
14. **Li, X., D. Qin, G. Jiang, K. Duan, and H. Zhou.** 2003. Atmospheric pollution of a remote area of Tianshan Mountain: ice core record. J. Geophys. Res. **108(D14):**4406–4416.
15. **Liu, Y., T. Yao, N. Jiao, S. Kang, B. Xu, Y. Zeng, S. Huang, and X. Liu.** 2009. Bacteria diversity in the snow over Tibetan plateau glaciers. Extremophiles **13:**411–423.

16. **Liu, Y., T. Yao, S. Kang, N. Jiao, Y. Zeng, S. Huang, and T. Luo.** 2007. Microbial community structure in major habitats above 6000 m on Mount Everest. Chin. Sci. Bull. **52:**2350–2357.
17. **Lozupone, C., and R. Knight.** 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. **71:**8228–8235.
18. **Miteva, V. I., P. P. Sheridan, and J. E. Brenchley.** 2004. Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl. Environ. Microbiol. **70:**202–213.
19. **Mueller, D. R., and W. H. Pollard.** 2004. Gradient analysis of cryoconite ecosystems from two polar glaciers. Polar Biol. **27:**66–74.
20. **Noble, P. A., P. E. Dabinett, and J. Crow.** 1990. A numerical taxonomic study of pelagic and benthic surface-layer bacteria in seasonally-cold coastal waters. Syst. Appl. Microbiol. **13:**77–85.
21. **Phung, N. T., J. Lee, K. H. Kang, I. S. Chang, G. M. Gadd, and B. H. Kim.** 2004. Analysis of microbial diversity in oligotrophic microbial fuel cells using 16S rDNA sequences. FEMS Microbiol. Lett. **233:**77–82.
22. **Skidmore, M., S. P. Anderson, M. Sharp, J. Foght, and B. D. Lanoil.** 2005. Comparison of microbial community composition in two subglacial environments reveals a possible role for microbes in chemical weathering processes. Appl. Environ. Microbiol. **71:**6986–6997.
23. **Stibal, M., M. Sabacka, and K. Kastovska.** 2006. Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. Microb. Ecol. **52:**644–654.
24. **Takeuchi, N., and S. Koshima.** 2004. A snow algal community on a Patagonian glacier, Tyndall glacier in the southern Patagonia Icefield. Arct. Antarct. Alpine Res. **36:**91–98.
25. **Takeuchi, N., J. Uetake, K. Fujita, V. Aizen, and S. Nikitin.** 2006. A snow algal community on Akkem Glacier in the Russian Altai Mountains. Ann. Glaciol. **43:**378–384.
26. **Takeuchi, N.** 2002. Surface albedo and characteristics of cryoconite on an Alaska glacier (Gulkana Glacier in the Alaska Range). Bull. Glaciol. Res. **19:**63–70.
27. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:**1596–1599.
28. **Tamura, K., M. Nei, and S. Kumar.** 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. USA **101:**11030–11035.
29. **Taton, A., S. Grubisic, E. Brambilla, R. De Wit, and A. Wilmotte.** 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl. Environ. Microbiol. **69:**5157–5169.
30. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The CLUSTAL-X Windows interface—flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24:**4876–4882.
31. **Vincent, W. F.** 2000. Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarct. Sci. **12:**374–385.
32. **Xiang, S. R., T. D. Yao, G. J. Wu, Y. Chen, T. C. Shang, L. L. Pu, and L. Z. An.** 2006. Deposition properties of bacterial populations in the Muztag Ata ice core. Quat. Sci. **26:**185–191.
33. **You, X. N., Q. Li, F. Wang, and Y. Zhu.** 2006. Seasonal evolution of insoluble microparticles stratigraphy in glacier no. 1 percolation zone, Eastern Tianshan, China. Adv. Earth Sci. **21:**1164–1170.
34. **Zhang, S., S. Hou, X. Ma, D. Qin, and T. Chen.** 2007. Culturable bacteria in Himalayan glacial ice in response to atmospheric circulation Biogeoscience **4:**1–9.
35. **Zhang, X., T. Yao, and X. Ma.** 2002. Microorganism in a high altitude glacier ice in Tibet, China. Folia Microbiol. **47:**241–245.
36. **Zhang, X., X. Ma, N. Wang, and T. Yao.** 2009. New subgroups of *Bacteroidetes* and diverse microorganisms in Tibetan plateau glacial ice provide a biological record of environmental conditions. FEMS Microbiol. Ecol. **67:**21–29.
37. **Zhang, X. F., T. D. Yao, S. J. Xu, and L. Z. An.** 2008. Phylogenetic and physiological diversity of microorganisms isolated from Puruogangri ice core. Microb. Ecol. **55:**476–488.
38. **Zhou, J., M. A. Bruns, and J. M. Tiedje.** 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. **62:**316–322.